REMARKS

Claim 1 has been amended to specify that the antigen has not been structurally changed and is not incorporated into the VSSPs. Support for the former limitation can be found at, for example, paragraphs 17 and 21 of the published application. Support for the latter limitation can be found at, for example, paragraphs 54 and 60 of the published application.

Applicants submit that the above amendments do not add any new matter, and their entry is requested.

Summary of the Present Invention

The present invention is directed to a pharmaceutical composition that potentiates the immunogenicity of low immunogenic antigens. The composition comprises the low immunogenic antigens and a vaccine carrier. The vaccine carrier consists of very small size proteoliposomes (VSSPs). The VSSPs are derived from the Outer Membrane Protein Complex (OMPC) of *Neisseria meningitidis* and include gangliosides that have been incorporated into the OMPC. The low immunogenic antigens are selected from the group consisting of peptides, polypeptides, proteins and their corresponding nucleic acid sequences. The antigens have not been structurally modified, i.e., they remain in the native form, and have not been incorporated into the VSSPs. The vaccine carrier stimulates and potentiates the immune response against the low immunogenic antigen. Both the humoral immune response and the cellular immune response are stimulated and potentiated by the vaccine carrier. As noted, the immune response is against the low immunogenic antigen which is a peptide, etc. as set forth in the claims. The potentiation of the cellular immune response includes a potentiation of the induction ability of cytotoxic T cells, such as $CD8^+$ T cells as shown in Example 18.

First Rejection Under 35 U.S.C. § 103(a)

The Examiner has rejected claims 1, 3-11, 27 and 29 under 35 U.S.C. § 103(a) as being obvious over Rodriguez et al. (US 5,788,985) in view of Estevez et al. (*Vaccine* **18**:190-197, 2000), Hammonds et al. (US 4,857, 637) and Udayachander et al. (*Human Antibodies* **8**:60-64, 1997). The Examiner cites Rodriguez et al. '985 for its disclosure of a pharmaceutical composition comprising the OMPC of *Neisseria meningitidis* into which gangliosides, especially N-glycoyl GM3, has been incorporated (i.e., VSSPs). The Examiner further cites Rodriguez et al. '985 for its disclosure that the pharmaceutical composition increases the immune response against N-gylcolated ganglioside which can be used for treating cancer, especially breast cancer which has a higher expression of gangliosides GM3 and GD3. The Examiner concludes that Rodriguez et al. teach that gangliosides are targets in treatment approaches. The Examiner notes that Rodriguez et al. does not teach that the pharmaceutical composition further comprises a low immunogenic antigen (such as HER-1) or an adjuvant (such as Incomplete Freund's adjuvant) and that Rodriguez et al. does not teach that the pharmaceutical composition stimulates both humoral and cellular responses against the low immunogenic antigen.

The Examiner contends that the newly cited Estevez et al. discloses that the VSSPs stimulates cellular and humoral immune responses. Specifically, The Examiner contends that Estevez et al. discloses that immunization using OMPC with incorporated gangliosides results in significant levels of T-dependent IgG1, IgG2a and IgG2b demonstrating a cellular immune response, as well as significant levels of T-independent IgG3 and IgM demonstrating a humoral response immune response. The Examiner cites the abstract for the disclosure that VSSPs overcame the natural tolerance to low-immunogenic self-antigen gangliosides in an adjuvant-dependent fashion. The Examiner further cites page 196 for the disclosure that it was known that the serotype proteins that are the main components of the OMPC induce proliferation and activation of lymphocytes and lead to the secretion of IL-2. The Examiner also states that Estevez et al. discloses that mice immunized with VSSPs derived from the OMPC of *Neisseria meningitidis* with gangliosides

incorporated therein in combination with Montanide ISA 51 resulted in increased immunoglobulin titres compared to mice immunized with VSSPs without Montanide ISA 51.

The Examiner cites Hammonds et al. for its disclosure of using EGFR (i.e., HER-1) as an antigen to immunize animals and for its disclosure that EGFR is overexpressed in malignant cells and thus is a desirable target for therapy. Hammonds et al. also discloses the use of an adjuvant for immunization with growth factor receptors.

The Examiner cites Udayachander et al. for its disclosure that many malignancies, such as breast cancer overexpress EGFR and that EGFR is a target for therapy.

The Examiner states that these references teach the importance of each of the claimed pharmaceutical composition components in stimulating an immune response to ganglioside or EGFR antigen. The Examiner also states that the references are deficient in that they do not teach using these components together. However, she concludes that it would be *prima facie* obvious to use the OMPC of *Neisseria meningitidis* into which ganglioside antigens have been incorporated as taught by Rodriguez et al. '985 and Estevez et al. and the EGFR antigen of Hammonds et al. in order to treat malignant tumors that overexpress these two antigens, such as breast cancer, because Rodriguez et al. '985 and Udayachander et al. teach that breast cancer overexpresses these antigens. The Examiner contends that it would have been obvious to combine two modes of treatment since the idea of combining them flows logically from each mode having been taught in the prior art. The Examiner contends that a skilled artisan would have reasonably expected to obtain effective therapeutic targeting of malignant tumors because both antigens were shown to elicit an immune response. The Examiner also contends that it would have been obvious to use an adjuvant in conjunction with the two antigens in view of the teachings of Hammonds et al. and Estevez et al. Although the Examiner notes that the references do not teach the stimulation of both humoral and cellular immune response, she contends that the composition taught by the prior art would necessarily induce both immune responses in view of the teaching in Estevez et al. that VSSPs are known to induce humoral and cellular immune responses against low-immunogenic self-antigens.

In response to Applicants prior arguments that Rodriguez et al. '985, as well as Hammonds et al. and Udayachander et al., did not disclose that the VSSPs could potentiate the immunogenicity of the low immunogenic antigens and thus did not teach the adjuvant properties of VSSPs, the Examiner contends in item 5 of the outstanding Office Action that Applicants are incorrect in this analysis. Specifically, the Examiner contends that the prior art does teach the adjuvant property of VSSPs for poorly immunogenic antigens and that this was a known characteristic of VSSPs citing Estevez et al. See the Examiner's comments in the paragraph bridging pages 10 and 11. In addition, the Examiner contends that Estevez et al. teaches that VSSPs, which are derived from OMPC of *Neisseria meningitidis*, are known to induce humoral and cellular immune responses against low immunogenic self-antigens. Thus, she contends that the composition suggested by the prior art, i.e., the composition of the two components, necessarily induces both a humoral and a cellular response.

In response to Applicants prior arguments that Applicants surprisingly found that the claimed pharmaceutical composition immunogenicity to low immunogenic peptides and that use of VSSPs as an adjuvant is not disclosed in the prior art, the Examiner notes that VSSPs were known in the art to potentiate the immunogenicity of low immunogenic antigens (i.e., apparently the gangliosides that were added to the OMPC) and to stimulate both humoral and cellular immune responses and hence this is not a surprising effect. The Examiner contends that this effect was known at the time of filing. She further contends that this position is supported in the art with her citation of Livingston et al. (*Vaccine* **11**:1199-1204, 1993) and Levi et al. (*Vaccine* **13**:1353-1359, 1995). The Examiner notes that Livingston et al. discloses that proteosomes from the outer membrane proteins of *Neisseria meningitidis* have been shown to initiate or augment IgG antibody responses against peptides when hydrophobically complexed to them. The Examiner further notes that Levi et al. discloses that proteosomes prepared from *Neisseria meningitidis* with influenza peptide antigens hydrophobically incorporated generated both humoral and cellular response to the antigens and that the proteosomes act as a carrier and adjuvant for peptide-based vaccines.

Applicants submit that the Examiner is in error in this rejection, particularly with respect to the teachings of the prior art as more fully detailed below.

Reply to First Rejection Under 35 U.S.C. § 103(a)

As Applicants have previously discussed and as the Examiner recognizes, Rodriguez et al. '985 does not teach or suggest that VSSPs act as an adjuvant to render immunogenic any further peptide, protein or polypeptide like HER-1 that is in a composition comprising the peptide and VSSPs. Applicants submit that all that Rodriguez et al. teaches is that VSSPs can be used for the treatment of certain types of cancer if NGcGM3 or NAcGM3 gangliosides are present as tumor-associated antigens in these tumors. It is the feature of the present invention that Applicants have discovered that VSSPs act as an adjuvant for low immunogenic antigens in which such antigens are peptides, polypeptides, proteins and their corresponding nucleic acid sequences.

Applicants note that Estevez et al. is the paper reporting the results used in Rodriguez et al. '985. Similar to Rodriguez at al., Estevez et al. only teaches how the hydrophobic insertion in the OMPCs outer lipid layers of an amphypatic molecule by nature like gangliosides are good enough to make immunogenic such poorly-immunogenic entities. Thus, Applicants submit that Estevez et al. is not really different from Rodriguez et al. '985 in that both documents referred exactly to the same type of vehicles (VSSPs) and to the same type of technical solution **to one particular problem**, making conveniently immunogenic two important targets for cancer therapy, the GM3 gangliosides.

Applicants submit that this fact is important because the Examiner appears to have over generalized the teachings of Estevez et al. In particular, Estevez et al., like Rodriguez et al. and even Levi et al., teaches that for making immunogenic any selected antigen this molecule should be naturally or synthetically modified to posses a hydrophic tail by which it **will necessarily be inserted** into the lipid layer of the OMPCs of *Neisseria meningitidis*. Thus, the hydrophobic tails of the GM3, NGcGM3 and GD3 gangliosides of Rodriguez et al. '985 and Estevez et al. are inserted into the lipid layer of the OMPCs to increase the immunogenicity of the gangliosides. Similarly, the hydrophobic tails of the GD3 ganliosides of Livingston et al. are inserted into the lipid layer of the

OMPCs to increase the immunogenicity of the gangliosides. On the other hand, Levi et al. teaches that peptide antigens are structurally modified to include a hydrophobic tail. These modified peptide antigens are inserted into the lipid layer of the OMPCs in order to increase the immunogenicity of the peptide antigen. These references only teach that the insertion of the low immunogenic antigens, gangliosides (Rodriguez et al. '985, Estevez et al. and Livingston et al.) or structurally modified peptide antigens (Levi et al.), into the OMPCs renders the specific antigens more immunogenic. It is the insertion of gangliosides into OMPCs which produces VSSPs. There is no teaching in any of these references that VSSPs have any adjuvant capability with respect to low immunogenic antigens which are not structurally modified or which are not incorporated into the VSSPs.

Totally different is the case of VSSPs as vehicles for peptides, polypeptides or proteins as antigens because it is sufficient to only mix the intact, non-modified peptide molecule with the VSSP preparation at the moment of the injection **(i.e., a "bed-side" adjuvant)** to produce a composition in which the immunogenicity of the unchanged peptide, etc. is potentiated. The peptides do not need to be incorporated into the VSSPs, such as by structurally modifying the peptides to contain a hydrophobic tail as taught by Levi et al. Any person skilled in the art will recognize the clear advantage of the solution to potentiating the low immunogenicity of peptides of the present invention over previously described solutions, such as set forth in Rodriguez et al. '985, Estevez et al., Livingtson et al. and Levi et al. The fact that the natural protein structure is not modified in any form in the present invention first keeps it antigenically "virgin" for immune cells and secondly means that any peptide molecule can be used. This is not the case for the OMPCs technology which requires chemical modification of the peptide antigen as taught by Levi et al. or which requires incorporation into the OMPCs as taught by Rodriguez et al. '985, Estevez et al. and Livingston et al. As it is well known in the art, such chemical modification usually renders neo-antigens with important changes in immune dominance, **basically for the $TCD8^+$ effector cells repertoire**, hence limiting the antigenic universe that can be used. Applicants submit that the amendment of the claims to specify that the antigen is not structurally changed or incorporated into the VSSPs clearly distinguishes the present invention from the teachings of the prior art. The fact

that the VSSPs are able to act as an adjuvant for low immunogenic peptide antigens without incorporating the peptide antigen into the lipid layer of the VSSPs or without structurally modifying the peptide antigen is not suggested by the cited prior art, and indeed is unexpected from the teachings of the prior art which teaches away from this finding by Applicants.

With respect to the Examiner's "reading" of Estevez et al., Applicants first note that this reference talks about anti-ganglioside humoral response, showing that VSSPs can induce anti-GM3 IgG antibodies. Again it is noted that the VSSPs in this Estevez et al. are OMPCs into which the low immunogenic gangliosides have been incorporated. The induction of anti-GM3 IgG antibodies simply means **the involvement of $CD4^+$ T helper cells** for this response. The same response is obtained for the serotype proteins of OMPCs because the meaning of lymphocyte stimulation and IL2 secretion is only related to $CD4^+$ T helper cells. Estevez et al. didn't provide evidences or even any suggestion concerning VSSPs activation of $CD8^+$ T effector cells, which are generally accepted by the skilled artisan as being more effective for cancer immunotherapy. Indeed the finding of this immune potentiating effect of VSSPs is **the basic surprising property** of the present invention, i.e., it shows how VSSPs can promote effective anti-HER-1 associated anti-metastatic effects which are dependent on $CD8^+$ T effector cells. This property of potentiating the induction of $CD8^+$ T effector cells by VSSPs is shown in Example 18 of the present application. This finding of the present invention has been confirmed and has been published in peer-reviewed journals. *See*, Sanchez-Ramírez et al. (*Int J Cancer* **119**:2190-2199, 2006; copy submitted with Amendment After Final dated 3 April 2007) for the anti-metastatic immunotherapy of Lewis lung carcinoma using a composition comprising EGFR (i.e., HER-1) and VSSPs, the latter as an adjuvant. *See also*, Torréns et al. (*Vaccine* **23**:5768-5774, 2005; copy submitted with Amendment After Final dated 3 April 2007) for the immunotherapy of HPV type 16 E-7 expressing tumors using a composition comprising CTL peptide and VSSPs, the latter as an adjuvant.

Hammond et al. discloses a method for selecting immunogens from a group of growth hormone factors and EGFR was included as an example. These disclosed immunogens are designed only for specific agonist antibody production in the inoculated animals. The reasoning of these

authors is to associate the induction of agonist anti-EGFR antibodies in the host with an effective therapeutic effect against, for example, HER-1 positive tumours. The recent failure of the Pharmexa's HER-2 conjugated vaccine, which aim was only to induce anti-HER-2 polyclonal antibodies in breast cancer patients, obtaining similar advantages as the passive administration of Herceptin, reinforces the concept that only the active induction of antibodies in a cancer patient will not be enough to achieve an impact over the disease.

Hammond et al.'s solution is a formulation composed of a fragment or the entire receptor domain molecule, **necessarily covalently coupled to an immunogenic protein carrier (claim 2) (KLH)** and Freund's adjuvant. This solution is very close to Pharmexa's vaccine and again is different and less general than the solution provided in the present invention. Teachings from Hammond et al. will not be used by skilled artisans for the induction of, e.g., strong anti-HER-1 $CD8^+$ T effector cell immunity.

Applicants understand that the basis for the Examiner's ***prima facie*** argument of "**obvious from the prior art**" is the consideration that the prior art suggests **a combined vaccine solution** (gangliosides and EGFR as antigens). Such a combined vaccine solution would claim the obvious advantages of obtaining antibody responses against gangliosides and HER-1, simultaneously, for the treatment of tumours. However, the present invention is not based on the combination of two antigens or the original discovery that OMPCs with inserted gangliosides potentiate the immunogenicity of the inserted gangliosides, but is based on the **surprising finding** that VSSPs are able to function as an adjuvant for low immunogenic peptide antigens. This adjuvant property of VSSPs is neither taught nor suggested in the prior art.

In VSSP technology, GM3ganglioside plays a key role in the nanoparticulated character of the vesicles, which in turn is a basic factor for its quality as a vehicle for vaccines, i.e., an adjuvant. The main achievement of VSSP adjuvant technology is its special ability in the promotion, e.g., induction, of $CD8^+$ T effector cells, specific for the accompanying protein or polypeptide, **without** the necessity of modifying the original structure of the peptide antigen or the incorporation of the peptide antigen into the VSSPs, i.e., the lipid layer of the OMPCs. Simply mixing VSSPs with the

peptide antigen before injecting it to the host is all that is required to achieve the potentiation of the immune response to the peptide antigen, including the potentiation of the effect on $CD8^+$ T effector cells.

It has been found that the HER-1/VSSP vaccine is anti-metastatic in a tumour mouse model while at the same time VSSP alone is inactive. *See*, the attached figure. This figure shows the results of an experiment in which mice (10 per group) were immunized SC, 3 times (once each 14 days), with either DEC-REGFm in VSSP or just with VSSP and buffer. 200,000 3LL D122 Lewis lung carcinoma cells were inoculated in mice right footpads 13 days after the first vaccination. Twenty one days later primary tumours were removed by surgery and then after 21 days more animals were sacrificed , their lungs removed and weighed. (A) Average lung weights in the two groups are compared in A. Results of the same experiment but depleting $CD8^+$ T cells after completing the vaccination schedule and before surgery are shown in B. This finding, as illustrated in the attached figure, indicates that the value of the present invention, i.e., the generation of a humoral and cellular immune responses to a low immunogenic peptide antigen, conferred by the adjuvant effect of VSSPs on the peptide antigen. That is, the humoral and cellular immune response to a low immunogenic peptide antigen is stimulated and potentiated by the adjuvant effect of VSSPs on the peptide antigen.

Applicants note that VSSPs are definitively a superior solution in the therapeutic vaccine field, such as applied to the HER-1 antigen. Such a superior solution is the aim of the present invention. Moreover, as evidence that this is a technical solution for peptides or proteins different from HER-1, the Examiner's attention is directed to Torréns et al. which describes the effect of VSSP in a vaccine comprising peptides derived from the human papilloma virus. As disclosed in the present application, these peptides have not been structurally modified for incorporation into the VSSPs.

In view of the above amendments and remarks, it is submitted that the claimed subject matter is not obvious from the teachings of Rodriguez et al. '985 in view of Estevez et al., Hammonds et al. and Udayachander et al. Withdrawal of this rejection is requested.

Second Rejection Under 35 U.S.C. § 103(a)

The Examiner has rejected claims 1, 3-11 and 27-29 under 35 U.S.C. § 103(a) as being obvious over Rodriguez et al. (US 6,149,921) in view of Estevez et al., Hammonds et al. and Udayachander et al. The Examiner's reasons for this second rejection are, in essence, the same as for the first rejection. The primary difference is that Rodriguez et al. '921 is cited for the additional disclosure of VSSPs with N-acetylated gangliosides.

In response to Applicants prior arguments concerning N-acetylated gangliosides versus N-glycolated gangliosides, the Examiner notes that Rodriguez et al. '921 addresses these arguments.

Applicants submit that the Examiner is in error in this rejection, particularly with respect to the teachings of the prior art as more fully detailed above.

Reply to Second Rejection Under 35 U.S.C. § 103(a)

As noted above, Rodriguez et al. '921 was cited for the incorporation of N-acetylated gangliosides into the OMPCs of *Neisseria meningitidis* to produce VSSPs which potentiated the immunogenicity of the incorporated N-acetylated gangliosides. Other than the use of N-acetylated gangliosides, the teachings of Rodriguez et al. '921 are the same as the teachings of Rodriguez et al. '985. Thus, the same comments made above concerning Rodriguez et al. '985 are also fully applicable to Rodriguez et al. '921. The same comments made above with respect to the remaining cited references are fully applicable to this rejection as well. Accordingly, it is submitted that the cited prior art does not teach or suggest the surprising effects found by Applicants for the presently claimed pharmaceutical compositions for the reasons discussed above.

In view of the above amendments and remarks, it is submitted that the claimed subject matter is not obvious from the teachings of Rodriguez et al. '921 in view of Estevez et al., Hammonds et al. And Udayachander et al. Withdrawal of this rejection is requested.

Concluding Remarks

In view of the above amendments and remarks, it is believed that the present claims satisfy the provisions of the patent statutes and are patentable over the cited prior art. Reconsideration of the application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned to expedite the prosecution of the application.

Respectfully submitted,

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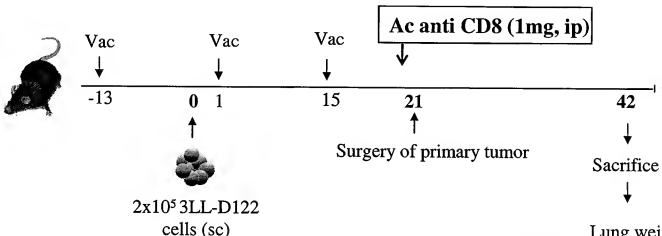
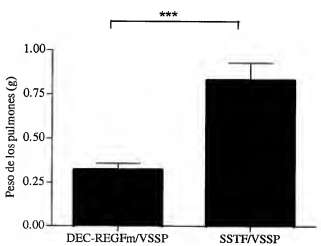
ATTACHMENT: Figure showing adjuvant effect of VSSPs.

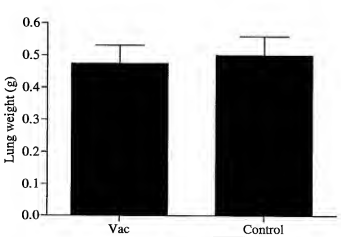
Vaccination with mEGFR-ECD/VSSP has anti-metastatic effect

Spontaneous metastasis/3LL-D122 model





Student t test; p<0,0001



Student t test; p>0,05

DEC-REGF; EGF receptor Extracelular domain
SSTF; PBS (phosphate buffer solution)